ELECTROMOBILITY FOCUSING CONTROLLED CHANNEL ELECTROPHORESIS SYSTEM

BACKGROUND

1. Field of the Invention

The invention relates to analytical procedures for investigating analyte species in a fluid sample. More specifically, the invention relates to methods and apparatus for separation and manipulation of analytes, and their application in diagnostic qualitative and quantitative procedures.

2. Brief Description of Related Art

Electrormigration separation processes, such as capillary zone electrophoresis and micellular electrokinetic capillary chromatography and other similar processes, are known and are widely used. For example, these processes are used in research and in various testing applications such as separations of proteins and DNA fragments. Control, and particularly fine control, of these processes is an-area of ongoing research and development efforts, as manipulation of quantities of analyte species for separation and qualitative and quantitative analysis is recognized as a significant technical challenge, but one potentially yielding great benefits in scientific endeavors and applications in areas such as healthcare that could potentially greatly benefit humankind.

Taking as an example capillary electrophoresis (CE), and with reference to FIG. **1** which is a generalized schematic diagram of CE, a capillary tube **12** having a longitudinal axis is disposed between two fluid wells positioned at its ends. An electrolyte solution, such as a buffer solution, is contained in the wells and an interior channel defined by the capillary. A cathode **14** is positioned in fluid communication with the electrolyte solution at one end and the anode **16** at the other. An applied voltage potential across the cathode and anode gives the potential or voltage profile **20** shown in the diagram. This is a straight line having a slope equal to the magnitude of the potential difference over the length of the channel. The electric field **22** created in the capillary is a constant throughout its length as shown. This of course is because the channel is of uniform cross-section and the voltage drop per unit of distance along the longitudinal axis of the channel is a constant value. In other words, the derivative of the function defined by the voltage as a function of position along the axis is a constant and, accordingly, the field generated is also of a constant intensity along the longitudinal axis.

Moreover, as is known in electrophoretic processes, an electrolyte solution, which provides a medium in which analytes to be investigated, e.g. separated and identified, are resident during separation, can be subject to electroosmotic flow, and such flow in small channels is a plug or bulk flow. Further, it has been recognized that electroosmotic bulk flow of electrolyte fluid in electrophoretic processes can be used to enhance separation, and also to do other things, such as to move analytes around within a electrophoretic separation apparatus. For example, U.S. Pat. No. 5,151,164, U.S. Pat. No. 5,180,475 and U.S. Pat. No. 5,320,730 disclose methods and apparatus for controlling the electroosmotic bulk flow of electrolyte solutions in electrophoretic processes, and the disclosures of these patents are hereby incorporated herein by reference.

It is known that control of the polarity and magnitude of charge accumulation adjacent to the inner surface of containments (such as fused silica capillary tubes) wherein the electrolyte solution dwells during electrophoretic separations (known in the art as the zeta potential) effectively controls electroosmotic bulk flow in small channels. This has been explained in terms of viscous coupling of molecules in solution with accumulations of charged molecules adjacent to the inner surface, such accumulated charged molecules being actuated in a longitudinal direction toward a cathode or anode (depending on polarity) by the electric field within the containment. As mentioned, this effect has been found to create a reproducible "plug" flow of electrolyte solution, and that this flow is relatively stable. Thus, in electrophoretic separation processes, analytes may be carried along in the bulk flow of the electrolyte solution, which fact can be used to move analytes, and assist in the separation of distinct analyte species based on differing mobilities of constituent molecules in the electrolyte solution (and any other media, such as a gel, which also may be present) within the separation channel.

For example, it is known that resolution of discrete analyte species having similar mobilities is enhanced by balancing electroosmotic flow against electrophoretic migration. Depending on whether cations or anions are of interest, the polarity and magnitude of the zeta potential is selected by applying an appropriate potential at the outer surface of a capillary having appropriate dielectric properties. Applying sufficient external potential of the same polarity as the molecules collecting at the surface overcomes the electroosmotic flow regime spontaneously occurring in the direction of electrophoretic migration by reversing the polarity of the inner surface, causing the spontaneously accumulating molecules to disperse and those of opposite polarity to migrate to the inner surface, setting up bulk flow in the opposite direction. As will be recognized, by application of appropriate potential external to the capillary, the electroosmotic flow within it can be increased, decreased, stopped and reversed.

However, manipulation of the electromagnetic forces effecting electrophoretic migration of charged species, which gives rise to separation, is less well recognized and understood as a tool in enhancing separation. Some work in this area has been done. For example, two published articles discuss localized modification of the intensity of the electric field for the purpose of improving resolution of analyte species. In an article [Kroegler and Ivory] published in [Journal of Chromatography A, 229 (1996) 229–236] there is a disclosure of a separation system wherein an electric field intensity which varies as a function of position, providing a sloping intensity profile along a longitudinal axis of the apparatus, is balanced against a pump-induced flow providing a counter-acting force. However, the system disclosed requires two flow fields, separated by a length of dialysis membrane tubing. One field comprises an electrolyte solution in which the electric field is propagated and is bounded by a trumpet shaped containment. The flared shape of the containment provides a cross section which varies as a non-linear continuous function of position along a longitudinal axis, and therefore gives an electric field within the channel that varies in intensity as a continuous function of position. Ions can cross the membrane, and the field extends within the other flow regime, but analyte molecules cannot pass out of the membrane into the electrolyte solution outside it. A buffer solution containing the analyte sample to be separated is pumped through the tubing in a direction opposite to the electrophoretic migration of the molecules of interest, and differences in electrophoretic mobility causes analyte species to "focus" at differing equilibrium points where the electrophoretic force balances the bulk flow force for each molecule of a species. In other words the molecules